(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 194 851**
A2

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: 86301729.9

(22) Date of filing: 11.03.86

(51) Int. Cl.⁴: **A61K 39/00** ,
//(A61K39/00,31:715)

(30) Priority: 11.03.85 US 710233

(43) Date of publication of application:
17.09.86 Bulletin 86/38

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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(54) **Human tumor therapy.**

(57) A pharmaceutical product for the treatment of human tumors comprising beta-(1-3)-glucan lentinan and, for subsequent administration, anti-tumor monoclonal antibodies which bind an antigen on the surface of human tumor cells and which have an isotype selected from IgG2a and IgG3.

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HUMAN TUMOR THERAPY

The invention described herein was made in the course of work under a grant or award from the Department of Health, Education and Welfare.

Technical Field

The present invention is directed to a therapeutic method for treating human tumors. More specifically, the present invention is directed to a method of treating human tumors with monoclonal antibodies in combination with lentinan.

Background of the Invention

In previous studies it has been shown that murine monoclonal antibodies (MAb) of IgG2a isotype that bind to human tumor cells specifically inhibit growth of the tumor cells in nude mice. Recently, a Mab of IgG3 isotype has also been shown to be effective. Herlyn et al., (1980) Cancer Res. 40:717-721; Herlyn & Koprowski, (1982) Proc. Natn. Acad. Sci. U.S.A. 79:4761-4765. There was evidence suggesting that tumor growth inhibition by the MAb probably was mediated by macrophages since treatment of nude mice with silica abolished the tumoricidal effects of the MAb. Furthermore, antibody-dependent macrophage-mediated cytotoxicity (ADMC) assays with human tumor cells in culture resulted in specific lysis of these cells. Thioglycollate-elicited murine peritoneal macrophages were used in these assays. Human macrophages have also been shown to lyse tumor targets coated with MAb. Steplewski et al., (1983) Science211:865-867. Macrophages, therefore, are strongly implicated as the effector cells mediating immunotherapeutic effects of, for example, MAb administered to gastrointestinal cancer patients. See, e.g., Koprowski in Proceedings of the IV Armand Hammer Cancer Symposium, pp. 17-38 (Boxx, Langman, Trowbridge & Dulbecco eds. 1984); Sears et al., (1984) J. Biol. Response Mod. 3:138-150.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method of tumor therapy.

Another object of the present invention is to provide a method of tumor therapy employing MAbs in which the therapeutic effects of the MAbs are enhanced.

Yet another object of the present invention is to provide a method of tumor therapy in which the therapeutic effect of MAbs is enhanced by the stimulation of macrophages.

These and other objects of the present invention are achieved by a therapeutic method for human tumors comprising:

administering to a tumor-bearing patient β-(1-3)glucan lentinan in an amount sufficient to stimulate macrophage activity; and

administering to said patient anti-tumor monoclonal antibodies after said lentinan administration, said monoclonal antibodies having an isotype selected from the group consisting of IgG2a and IgG3, and binding an antigen on the surface of said patient's tumor cells.

DESCRIPTION OF THE FIGURES

Figure 1 shows the effect of various lentinan dosages on ADMC reactivity of murine peritoneal macrophages against carcinoma SW1116 target cells, in the presence of IgG2a anti-colon carcinoma MAb. Curve A is at an effector to target cell ratio of 50; curve B is at an effector to target ratio of 10.

Figure 2 shows the kinetics of macrophage stimulation by lentinan. Macrophages were collected at various times after administration of lentinan to mice and assayed for ADMC reactivity with SW1116 target cells in the presence of anti-colon carcinoma MAb(o) (curve C). Minimal lysis was obtained in the presence of control anti-influenza virus MAb -(o) (curve D).

Figure 3 presents a comparison of the ADMC reactivity of lentinan-stimulated macrophages (solid lines on Figure) to thioglycollate-stimulated macrophages (dashed lines on Figure) in lysing melanoma target cells O, or colon carcinoma cells (●), in the presence of specific MAbs.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that the stimulation of macrophages invivo with β(1-3)glucan lentinan (hereinafter lentinan) renders them cytotoxic against tumor cells in vitro, in the presence of anti-tumor monoclonal antibodies of particular isotypes (Table 1).

Generally, the therapeutic method of the present invention comprises first administering lentinan to a tumor-bearing patient to stimulate macrophages, and then administering anti-tumor MAbs to the patient. Lentinan is a neutral polysaccharide whose physical and chemical properties are fully characterized. Briefly, it is isolated from a hot water extract from the fruit body of Lentinus edodes(Berk.) Sing. The chemical structure of lentinan is reported to be a β-1,3-glucan, with an average molecular weight distributed in the range between 4×10^5 and 8×10^5 daltons by gel permeation chromatography. According to elementary analysis, the molecular formula of lentinan is $(C_6H_{10}O_5)_n$. See generally, Chihara & Taguchi, (1982) Rev. Immunol. Immunopharmacol (Rome) 2:93-104.

The use of lentinan as a macrophage potentiator has been found to be preferred to other possible potentiators because it is a relatively safe compound to administer to patients. Furthermore, it is more effective than thioglycollate. Other agents, such as BCG and C. parvum, do not activate macrophages for tumor cell lysis by IgG2a MAbs.

The effectiveness of the therapeutic regimen of the present invention is dependent upon the timing and dosages of lentinan to the patient. Animal studies indicate that there is an optimal dose of lentinan with higher doses resulting in a decrease in macrophage activation. Other animal studies have indicated that the timing of lentinan administration is an important factor bearing upon the effectiveness of the therapy. Generally, optimal macrophage activation was observed from about 3 to about 5 days following the administration of lentinan. These time periods are based upon results of animal studies and may be varied somewhat as additional clinical data on humans is available. One skilled in the art, however, being aware that there is an optimal

dosage and that there are timing effects in animal studies will be able to establish an optimal dosage and timing of lentinan administration for human patients through routine clinical trials.

After a macrophage-stimulating quantity of lentinan has been administered to a patient, anti-tumor MAbs are administered to the patient; i.e., antibodies that bind an antigen on the surface of the patient's tumor cells. Desireably, the antibodies are administered at about the time that macrophage activation reaches a maximum, that is about 3 to about 5 days after lentinan activation. The MAbs should be of isotype IgG2a or IgG3, and preferably of isotype IgG2a. Macrophages stimulated by lentinan were not found to be cytotoxic for tumor cells coated with MAbs of isotype IgG2b, IgM, or IgA. Preferably, the MAbs are human MAbs.

The preparation of MAbs for immortal cell lines are well known in the art. For example, immortal, antibody-producing cell lines can be produced from normal B cells by hybridoma technology, Epstein-Barr virus transformation, or transformation with oncogenic DNA. See e.g., M Schreier et al., Hybridoma Techniques(Cold Spring Harbor Laboratory 1980); Hammerling et al., Monoclonal Antibodies and T-Cell Hybridomas (Elsevier Biomedical Press 1981); Kennett, et al., Monoclonal Antibodies (Plenum Press 1980); Kozbor et al., (1982) Proc. Natl. Acad. Sci. USA 79:6651-6655; Jonak et al., (1983) Hybridoma 2:124; Monoclonal Antibodies and Functional Cell Lines (Kennett, Becktol & McKearn eds. 1983); Kozbor et al., (1983) Immunology Today 4:72-79. The type of immortal cell line from which the MAbs are produced is not critical.

Those that are skilled in the art are familar with the use of MAbs in tumor therapy and the establishment of optimal dosages through routine clinical trials is well within the skill of the art. See e.g. Sears et al., J. Biol. Response Mod., 3:138-150 (1984). The examples below in mouse models will aid those skilled in the art in establishing optimum effective dosages and in timing dosages for both lentinan and anti-tumor MAbs in the treatment of human patients.

Pharmaceutical products are contemplated to carry out the anti-tumor therapy of the present invention. Such products comprise the two components, lentinan and anti-tumor monoclonal antibodies. The components should be kept separately, but may be packaged and sold as a kit or individually. The lentinan can be packaged in lyophilized form. The monoclonal antibodies can be packaged in a suitable physiological buffer, such as physiological saline, and should be kept frozen.

The lyophilized lentinan can be reconstituted into liquid form by dissolving in a suitable excipient such as sterile water, less than two weeks before intended use. Once reconstituted, the lentinan solution should be kept refrigerated and in the dark, as it is light sensitive.

Suitable pharmaceutical excipients for administration to human patients are well known in the art. The choice of an appropriate excipient is well within the skill of the clinician or pharmacist.

A suitable amount of the pharmaceutical product for a single dosage administration is between about 0.5 and 3.5 mg of beta-(1-3)-glucan lentinan and between about 100 and 500 mg of anti-tumor monoclonal antibodies.

Although applicants do not wish to be bound by this theory, it is believed that lentinan indirectly enhances anti-tumor cytotoxic effects of macrophages by direct activation of the alternate pathway of the complement system and/or by stimulating helper T-cells. The possible T-cell dependency is supported by the failure to find enhancing effects in athymic mice implanted with human tumors and treated with MAb.

The following examples are presented for illustrative purposes only and are not intended to limit the scope of the present invention.

MATERIALS AND METHODS

Human Tumor Cell Lines

Melanoma cell line WM-9, colorectal carcinoma cell line SW1116 and pancreatic carcinoma cell line Capan have been described. See Herlyn et al., (1983) Cancer Invest. 1:215-224; Koprowski et al., (1979) Somat. Cell. Genet. 5:957-971; Steplewski et al., (1979) Eur. J. Immunol. 9:94-96.

Murine MAbs

The MAbs included in this study are listed in Table 1. They were produced against colorectal carcinomas, melanomas and pancreatic carcinomas and have been described in detail previously. See Hansson et al., (1983) J. Biol. Chem. 258:4090-4097; Herlyn et al., (1983) supra; Koprowski et al., (1979), supra.

Murine Macrophages

Preparation of thioglycollate-elicited CBA macrophages adherent to wells of microtiter plates has been described. Lentinan-activated macrophages were obtained from 6-to 10-week-old CBA mice by intraperitoneal (i.p.) injection of 2.5 mg/kg body weight (BW) of lentinan (Ajinomoto Co., Tokyo, Japan) unless otherwise stated. Macrophages were collected at various times thereafter and plated as described for thioglycollate-elicited macrophages. See Herlyn and Koprowski (1982) Proc. Natl. Acad. Sci. USA 79:4761-4765. Thioglycollate and lentinan-stimulated adherent peritoneal cells consisted of 94% and 85% (mean of 3 experiments) macrophages, respectively, as determined by latex phagocytosis and non-specific esterase staining. Boltz-Nitulescu & Foerster, (1979) Immunology 38:621-630; Tucker et al. (1977) Journal of Immunological Methods 14 : 267-269. The cells contaminating the macrophages morphologically resembled fibroblasts and were non-phagocytic and esterase-negative.

ADMC Assays

The ADMC assay with [methyl-^{3}H]thymidine-labeled target cells was performed as described. See Herlyn & Koprowski (1982), supra. All ADMC values given are corrected for percent lysis obtained in the presence of anti-influenza virus control MAb.

Binding Assays

Binding of iodinated MAbs to Fc receptors on thioglycollate-or lentinan-activated macrophages was determined by adding to the adherent macrophages either various amounts of [^{125}I] MAb or constant amounts of [^{125}I] MAb mixed with increasing amounts of unlabeled MAb as

described. Unkeless et al., (1975) J. Exp. Med. 142: 1520-1533. Association constants of MAb binding and maximal number of binding sites per macrophage were determined by the method of Scatchard.

Statistical Analysis

Data were analyzed using the Student's t-test. A probability of less than 5% (p less than 0.05) was considered significant.

RESULTS

Effect of Various Lentinan Dosages on ADMC by MAb 17-1A

Between 0.25 and 5 mg lentinan per kg were administered to mice i.p.; ADMC reactivity of peritoneal macrophages against colorectal carcinoma cells SW-1116 coated with MAb 17-1A was assayed 3 days later at two different effector-to-target (E:T) cell ratios. As can be seen from Fig. 1, the ADMC levels were highest when lentinan was used at 2.5 mg/kg BW, and E:T cell ratio was 50. Whereas ADMC values increased over the entire dosage range at the lower E:T cell ratio of 10, these values were significantly (p less than 0.05) lower than those obtained at an E:T cell ratio of 50. Therefore, in the ADMC assays described below, macrophages were stimulated by injection of 2.5 mg lentinan per kg BW and E:T cell ratios of 50 were used. Increasing the E:T cell ratios above 50 did not result in higher ADMC values. Non-stimulated (resident) macrophages caused only 20% and 0% lysis in the presence of MAb 17-1A at E:T cell ratios of 50 and 10, respectively.

Kinetics of Macrophage Stimulation by Lentinan

Peritoneal macrophages were assayed for ADMC reactivity, 3, 5, 8 and 11 days following i.p. injection of 2.5 mg lentinan per kg BW. As can be seen from Fig. 2, ADMC activity of macrophages in the presence of specific MAb was highest 5 days following injection of lentinan, whereas values obtained in presence of control MAb did not differ on the various days tested. The increase in the percentage of non-phagocytic cells in the macrophage preparations from day 5 after the injection of lentinan might account for the decrease in macrophage activity with time. Therefore, macrophages were generally collected 3-5 days following the injection of lentinan.

Comparison of Lentinan and Thioglycollate-Stimulated Macrophages in ADMC

ADMC reactivity of lentinan-stimulated macrophages was compared with the reactivity of thioglycollate-elicited macrophages which have been used by us previously to demonstrate ADMC-reactivity of IgG2A MAbs. See Herlyn & Koprowski (1982), supra. Lentinan-activated macrophages showed higher lytic activities against colon carcinomas or melanomas coated with IgG2a MAbs as compared to thioglycollate-elicited macrophages (Fig. 3). These differences were significant (p less than 0.05) at all E:T cell ratios tested.

Comparison of MAbs of Various Isotypes in ADMC Assays with Lentinan-Stimulated Macrophages

ADMC-reactivities of MAbs produced against various human tumors and representing 6 different isotypes are presented in Table I. Lentinan-activated macrophages were used as effector cells. In these assays, all the IgG2a and IgG3 MAbs and some of the IgG1 MAbs were reactive whereas IgG2b, IgA and IgM MAbs were non-reactive.

TABLE 1

MAbs of Various Isotypes in ADMC with Lentinan-Stimulated Macrophases

MAB		Target		% Specific lysis[2]
Isotype	Code	Origin[1]	Code	Lentinan-macrophages
IgG1	ME8211	MEL	WM-9	0
	ME7771	MEL	WM-9	0
	ME529	MEL	WM-9	12.5
	19-9	CRC	SW1116	60.2
IgG2a	17-1A	CRC	SW1116	79.4
	ME377	MEL	WM-9	69.8
	ME5073	MEL	WM-9	42.4
	ME121	MEL	WM-9	24.6
IgG2b	ME3174	MEL	WM-9	0
	ME7965	MEL	WM-9	0
IgG3	PC2111	PC	Capan	34.0
	PC2195	PC	Capan	15.4
IgM	38a	CRC	SW1116	0
	ME919	MEL	WM-9	0
IgA	PC8352	PC	Capan	0

1. Abbreviations: CRC = colorectal carcinoma, MEL = melanoma, PC = pancreatic carcinoma.

2. Values represent means of triplicate determinations in two independently performed experiments. E:T cell ratios were 50. All values differed significantly ($p \leq 0.05$) from control values obtained with anti-influenza virus MAb. Values that did not differ from controls were designated zero.

Scatchard Analysis of MAb Binding to Murine Macrophages

Lentinan-and thioglycollate-stimulated macrophages bound 2.8 and 3.8 X 10^4 molecules of MAb 17-1A per macrophage, respectively. These values did not differ significantly (p less than 0.05). The association constants were 0.2 X 10^9 mole $^{-1}$ for both types of macrophages.

Since variations will be apparent to those skilled in the art, it is intended that this invention be limited only by the scope of the appended claims.

Claims

1. A pharmaceutical product for the treatment of human tumors comprising beta -(1-3)-glucan lentinan and, for subsequent administration, anti-tumor monoclonal antibodies which bind an antigen on the surface of human tumor cells and which have an isotype selected from IgG2a and IgG3.

2. A single dose of the product of claim 1 comprising between about 0.5 and 2.5 mg of beta-(1-3)-glucan lentinan and between about 100 and 500 mg of said anti-tumor monoclonal antibodies.

3. A pharmaceutical composition comprising beta-(1-3)-

glucan lentinan in an amount sufficient to stimulate macrophage activity for administration to a tumor-bearing patient subsequently to be treated with anti-tumor monoclonal antibodies binding an antigen on the surface of said patient's tumor cells and having an isotype selected from IgG2a and IgG3.

4. A pharmaceutical composition for administration to a tumor-bearing patient comprising an anti-tumor monoclonal antibody binding an antigen on the surface of said patient's tumor cells and having an isotype selected IgG2a and IgG3, when said patient has previously been treated with beta-(1-3)-glucan lentinan in an amount sufficient to stimulate macrophage activity.

5. A method of preparing a pharmaceutical composition for treatment of tumor-bearing patients comprising the steps of:

lyophilizing a beta-(1-3)-glucan lentinan preparation; and

preparing and freezing a solution containing anti-tumor monoclonal antibodies binding an antigen on the surface of said patient's tumor cells and having an isotype selected from IgG2a and IgG3 in an appropriate physiological buffer.

6. The pharmaceutical product or composition according to any of claims 1-5 in which the anti-tumor monoclonal antibodies have the isotype IgG2a.

7. The pharmaceutical product or composition according to any of claims 1, 2 and 3 in which the beta-(1-3)-glucan lentinan is for administration 3-5 days prior to administration of the anti-tumor monoclonal antibodies.

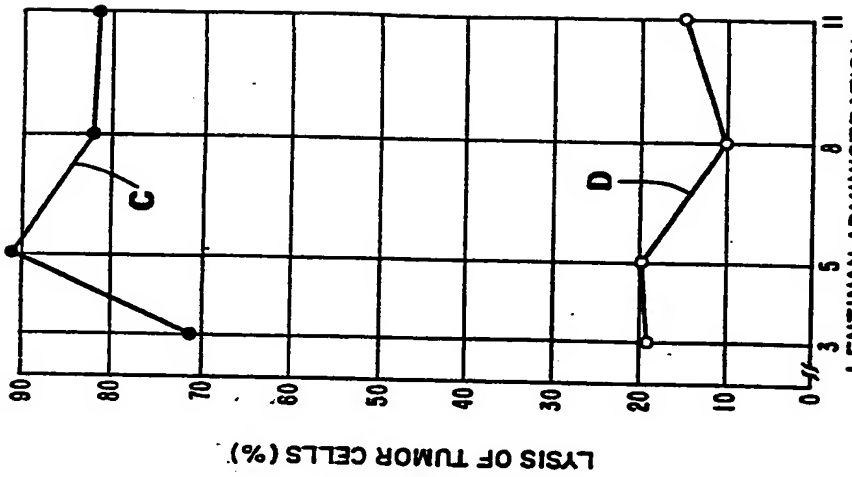
LYSIS OF TUMOR CELLS (%)
90
80
70
60
50
40
30
20
10
0
C
D
3
5
8
11
LENTINAN ADMINISTRATION
(DAYS BEFORE ASSAY)

FIG. 2

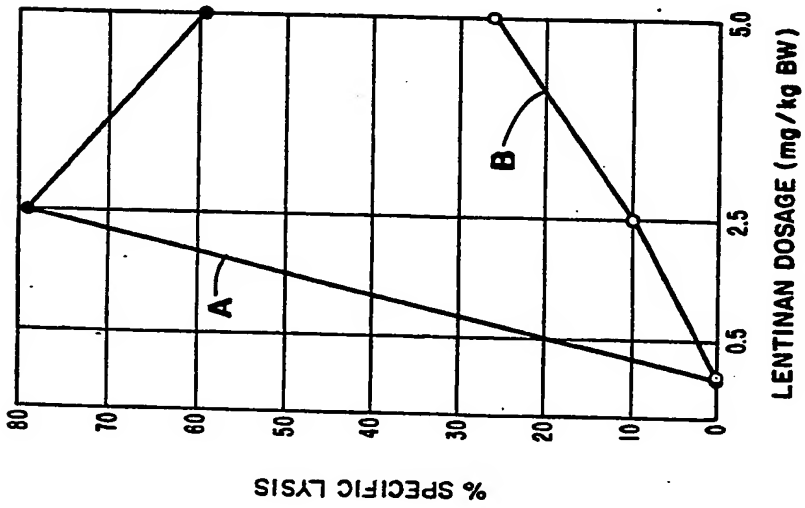
% SPECIFIC LYSIS
80
70
60
50
40
30
20
10
0
A
B
0.5
2.5
5.0
LENTINAN DOSAGE (mg/kg BW)

FIG. 1